## Erythropoiesis from human embryonic stem cells through erythropoietin-independent AKT signaling.

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**Public Summary:**
The tremendous ability of pluripotent stem cells (PSC) to generate diverse cell types has led to the possibility that these cells may one day be used as an inexhaustible supply of cells and tissue for therapy. One such potential application is as a source of red blood cells for transfusion. Human PSC can produce red cells in culture but the process is currently inefficient producing too few cells for practical use. In addition, the red cells produced are of an immature type, expressing mostly embryonic rather than adult hemoglobin. In this paper we found that if we introduced a synthetic “fusion” protein into PSC, we could produce significantly more red cells in culture and that those cells were also more mature. We also found that the synthetic protein acts to increase red cell production independently of the well known growth factor erythropoietin. Experiments revealed that the fusion protein activates unusual signaling pathways during PSC differentiation leading to changes in cycling and survival of the red cells. These studies reveal potentially new therapeutic targets for recovery from anemia and regeneration of blood cells for transplantation.

**Scientific Abstract:**
Unlimited self renewal capacity and differentiation potential make human pluripotent stem cells (PSC) a promising source for the ex vivo manufacture of red blood cells (RBC) for safe transfusion. Current methods to induce erythropoiesis from PSC suffer from low yields of RBCs, most of which are immature and contain embryonic and fetal rather than adult hemoglobins. We have previously shown that homo-dimerization of the intracellular component of MPL (ic-MPL) induces erythropoiesis from human cord blood progenitors. The goal of the present study was to investigate the potential of ic-MPL dimerization to induce erythropoiesis from human embryonic stem cells (hESC) and to identify the signaling pathways activated by this strategy. We present here evidence that ic-MPL dimerization induces erythropoietin (EPO)-independent erythroid differentiation from hESC by inducing the generation of erythroid progenitors and by promoting more efficient erythroid maturation with increased RBC enucleation as well as increased gamma:epsilon globin ratio and production of beta-globin protein. ic-MPL dimerization is significantly more potent than EPO in inducing erythropoiesis and its effect is additive to EPO. Signaling studies show that dimerization of ic-MPL, unlike stimulation of the wild type MPL receptor, activates AKT in the absence of JAK2/STAT5 signaling. AKT activation upregulates the GATA-1 and FOXO3 transcriptional pathways with resulting inhibition of apoptosis, modulation of cell cycle and enhanced maturation of erythroid cells. These findings open up potential new targets for the generation of therapeutically relevant RBC products from hPSC. Stem Cells 2014.